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BOTANICAL GAZETTE

SEPTEMBER 1910

SOME EFFECTS OF A HARMFUL ORGANIC SOIL CONSTITUENT¹

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(WITH ELEVEN FIGURES)

The investigation of infertile soils from various parts of the United States has received considerable attention in the last few years, and has been conducted along several converging lines in these laboratories. Among these is a thorough inquiry into the nature of the organic matter of soils. The results of these researches into the chemistry of the organic matter of the soil, its origin, transformation, and properties, have been reported upon to a large extent in a former bulletin of this Bureau and in scientific journals.² Several bodies have been isolated from such soils, which have quite different chemical properties, thus showing that there

¹ Published by permission of the Secretary of Agriculture.

² SCHREINER, O., and SHOREY, E. C., The isolation of dihydroxystearic acid from soils. *Jour. Amer. Chem. Soc.* 30:1599. 1908.

———, The isolation of picoline carboxylic acid from soils and its relation to soil fertility. *Jour. Amer. Chem. Soc.* 30:1295. 1908.

———, The presence of a cholesterol substance in soils; agosterol. *Jour. Amer. Chem. Soc.* 31:116. 1909.

———, A wax acid from soils; agroceric acid. *Science N.S.* 28:190. 1908.

———, Pentosans in soil. *Science N.S.* 31:308. 1910.

———, Purine bases in soils. *Science N.S.* 31:309. 1910.

———, The presence of secondary decomposition products of proteids in soils. *Proc. Amer. Soc. Biol. Chem.* 1:47. 1907.

See also Bull. 53, Bureau of Soils, U.S. Dept. Agric.

SCHREINER, O., and SULLIVAN, M. X., Soil fatigue caused by organic compounds. *Jour. Biol. Chem.* 6:39. 1909.

is a difference in the nature of the chemical bodies in different soils. Some of the bodies isolated contained nitrogen, while others free from this element contained carbon, hydrogen, and oxygen only. The presence of sulfur and phosphorus compounds has also been strongly indicated by these studies, although the actual identification of bodies of such nature has not been definitely accomplished. The results are sufficient, however, to show that a wide range of bodies of different composition exists in soils, and a more detailed knowledge of their nature and properties seems imperative in order to know and understand the nature of the material influencing crops in the field and the action of fertilizers upon these, either directly or indirectly, through the medium of root oxidation and reduction or by microbiologic and enzymotic influences.

In an earlier paper³ it was demonstrated that the roots of plants possessed a very appreciable oxidizing power. This oxidizing power of the normal root was found to be influenced by the medium in which it grew. Thus good soils and their aqueous extracts promoted oxidation, while the poorer soils hindered this function of the roots. Some substances harmful to plant growth were also found to have an inhibitive effect on this oxidation. When smaller amounts were present, however, it was found that the oxidizing power of the roots was able to overcome the harmful influence. An examination in one case, namely that of vanillin, where it was possible to demonstrate by colorimetric test the presence or absence of minute amounts, showed that this disappeared entirely from solution under the influence of the oxidizing power of the roots. It was further shown that the fertilizer salts, in addition to promoting plant growth, also had a very strong influence in promoting this enzymotic effect, this being especially marked in the case of sodium nitrate and lime.⁴ The action of fertilizer salts and the influence of such harmful soil constituents were further investigated, therefore, and the results given in connection with the

³ SCHREINER, O., and REED, H. S., Studies on the oxidizing power of roots. *BOT. GAZETTE* 47:355. 1909. See also *Bull.* 56, Bureau of Soils, U. S. Dept. Agric. 1909.

⁴ SCHREINER, O., and REED, H. S., The power of sodium nitrate and calcium carbonate to decrease toxicity in conjunction with plants growing in solution cultures *Jour. Amer. Chem. Soc.* 30:85. 1908.

other factors reported in this paper. All of the harmful bodies which had been studied and presented in previous papers had a distinct influence on the roots, causing them in many cases to become stunted or swollen and darkened at the tips, or to show other physiological irregularities of the same kind as is exhibited by the roots in different extracts from infertile soils. It is, therefore, of interest to know further the influence which such altered root conditions would have upon the composition of the soil solution and the influence of added fertilizers.

One of the bodies isolated from a number of unproductive soils is a definite crystalline body identified as dihydroxystearic acid melting at 98° – 99° C. It can be prepared by the oxidation of elaidic acid in the laboratory. In these experiments the dihydroxystearic acid used had been prepared in this manner. The frequent occurrence of this body in such soils and its disappearance therefrom by processes which promote aeration or oxidation made this especially suitable for further study of its effects on plant development in relation to the concentrations of soil solutions or fertilizer application.

The dihydroxystearic acid can be isolated from a soil containing it by treatment with a 2 per cent sodium hydroxid solution, and, after allowing the mineral material to settle, the alkaline extract is separated and made acid with a slight excess of acetic acid. The so-called humus precipitate which is thus formed is filtered off and the clear filtrate is shaken out with ether and the ether solution allowed to evaporate on the surface of a small quantity of water. The dihydroxystearic acid is left on the surface of the water, together with other impurities extracted by the ether. The impurities can be largely removed by heating the water to boiling, and filtering. Fig. 1 shows the effect of a solution of this nature on wheat seedlings when the material is dissolved in much water. The dihydroxystearic acid, when dissolved in a small volume of water and then cooled, crystallizes out in the form of small plates or leaflets arranged in radiating clusters. Fig. 2 shows the effect of the purified substance in various concentrations. The details of the method of isolating and purifying will be found in the paper cited.

Dihydroxystearic acid can also be prepared in the laboratory by starting with oleic acid, which by treatment with nitrous oxid is changed to the isomeric elaidic acid. The elaidic acid thus formed is dissolved in a solution of potassium hydroxid and oxidized by a solution of potassium permanganate, one of the products under suitable conditions being dihydroxystearic acid.

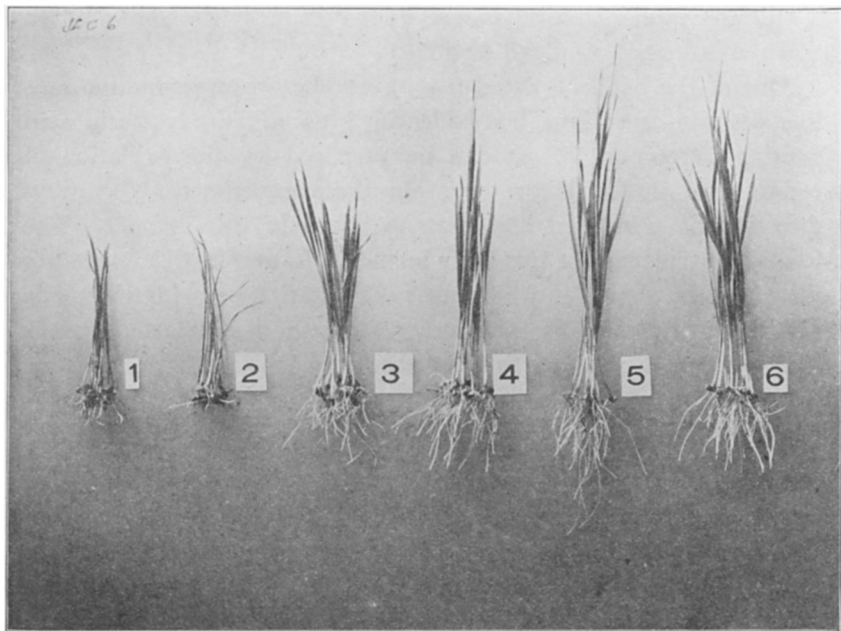


FIG. 1.—Wheat seedlings grown in extract obtained in the method for isolating dihydroxystearic acid from soils: 1, 2, undiluted extract; 3, 4, one part of extract, one part of distilled water; 5, 6, one part of extract, nine parts of distilled water.

Care has to be taken that the oxidation does not proceed too far, for the dihydroxystearic acid, as already mentioned above, is very readily oxidized to other compounds.

In this paper are reported experiments on the effect of a harmful soil constituent upon plant growth and upon soil solutions and fertilizer action, especially with reference to the ratio of phosphate, nitrate, and potash originally present and removed by wheat seedlings in the course of the experiment.

Solution cultures containing the three fertilizer ingredients, namely P_2O_5 , NH_3 , and K_2O , as calcium acid phosphate, sodium nitrate, and potassium sulfate, respectively, in all possible ratios of one, two, and three constituents, varying them in stages of 10 per cent, were prepared, the concentration being 80 parts per million in these constituents. In a similar set of cultures there

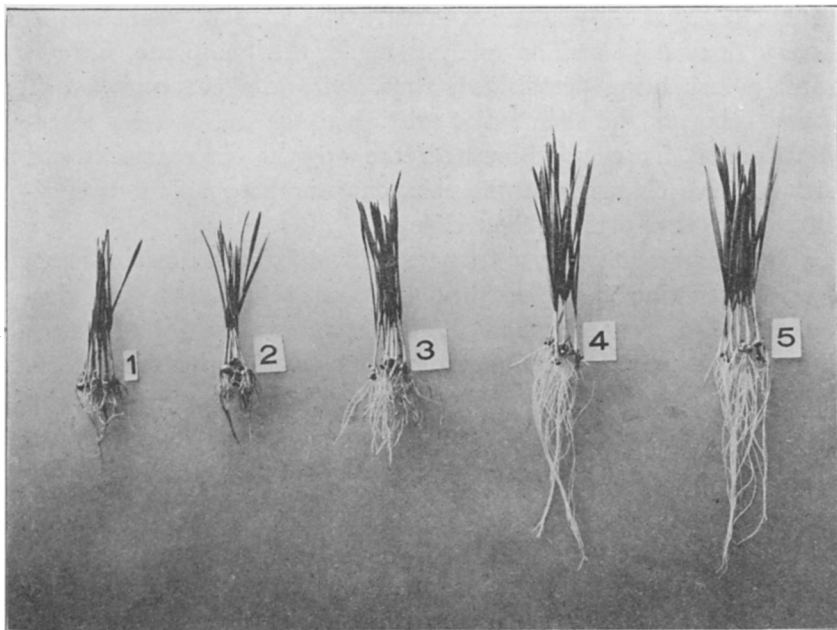


FIG. 2.—Wheat seedlings grown in solutions of dihydroxystearic acid from soils: 1, solution of dihydroxystearic acid, 200 parts per million; 2, 100 parts; 3, 50 parts; 4, 20 parts; 5, control in distilled water.

was added 50 parts per million of dihydroxystearic acid to each culture. The selection of the salts as carriers of the phosphate, nitrate, and potash, and the terms P_2O_5 , NH_3 , and K_2O are in harmony with the practice in fertilizer work. The salts selected, it will be seen, are also carriers of calcium, of sodium, and of sulfate, and the three salts, therefore, were practically the best that could be selected for giving at the same time other needed constituents. The details of this method of experimentation have been given in

an earlier paper, and the reader is referred to this for a full explanation of the use of the triangular diagram and the results obtained by growing seedling wheat in these various culture solutions without the presence of any added harmful substance.⁵ Wheat seedlings were then grown in these various cultures and observations made in regard to general development, the effect on the root growth and appearance, and on root oxidation, and at the termination of the experiment the green weight of the plants was taken. The solutions were changed every three days and an analysis made, the phosphate, nitrate, and potash being determined, thus giving the concentration of these elements and their ratios existing at the end of every three-day period for comparison with the original concentration and ratio. This changing of the solutions was kept up for twenty-four days, thus making eight changes.

In the present work a triangle of solution cultures similar to the one described in the previous paper was set up, with the difference that 50 parts per million of dihydroxystearic acid were present in each culture. This set of cultures grew from April 2 to April 26. The set without the dihydroxystearic acid grew from February 26 to March 21. While these two sets are not strictly comparable, owing to the fact that they were grown at different times, though under very similar greenhouse conditions and for the same length of time as well as in the same time of year, one closely following the other, the results show, nevertheless, very strikingly the effect of dihydroxystearic acid, and are duplicated or substantiated by two further experiments in which the sets were grown simultaneously.

The analytical results, however, were not so complete in these later tests, owing to the inability to handle the 396 separate determinations necessary every three days. The results given in this paper, therefore, are largely based on the first set, for the reason that the analytical results were more complete than in the other sets mentioned.

The difference between the cultures containing the dihydroxystearic acid and those without this substance was very marked,

⁵ SCHREINER, O., and SKINNER, J. J., Ratio of phosphate, nitrate, and potassium on absorption and growth. *BOT. GAZETTE* 50:1. 1910.

and was especially striking in those sets where both triangles of cultures were grown simultaneously. In addition to the general appearance of the tops, the presence of this harmful body produces still other effects readily recognized by the investigator, and forming on the whole a better physiological indicator of the toxicity than the growth of tops, as has been well recognized by physiologists generally in conducting similar work. Reference is here made to the action of the body on root condition and growth. The root being bathed by the solution, and being, moreover, the delicate mechanism of absorption, is often a more sensitive indicator than is the growth of the top. The most marked effects of dihydroxystearic acid on roots are strongly to inhibit their growth and to produce enlarged or swollen tips, which are frequently very dark in color and often turned back in the form of hooks. These phenomena are observed even when the injury is not so great as to kill the plants. This action of the body on the roots is influenced by the conditions of growth imposed upon the plant by the different fertilizer ratios. This is shown by the general notes taken on one of these sets one week after growth in the cultures had begun.

All the plants in the solutions having no nitrogen appear to be dying. The roots are already dead, have made little growth, and are much discolored, the tips are dark and swollen and a large number turned upward. The tops have made some growth, some of the leaves are curled, but most are still fresh and green.

The plants in the solutions containing no phosphate are similar to the above; the roots and tops are somewhat better, but still show an undoubted harmful action, the roots being dark and flimsy, tips swollen and often turned upward, the greater number being dead.

The plants in the solutions on the potash base line are better than the two sets described above. The upper parts of the roots of these plants are dark, the lower sections that have recently grown out are white and clear, but the tips are still dark and swollen, and many are turned upward.

The plants in the interior of the triangle, where all three fertilizer ingredients are present, are fairly good, with no great difference noticeable at this stage, but showing somewhat better plants near

the center. The roots, on the other hand, are already showing differences. They are rather poor along the line having 8 parts per million of NH_3 . The upper and older part of the roots is dark, the newer portion is white and clear, but the tips are swollen. The general condition of the roots, as a whole, is a great deal better than in case of those in the boundary lines of the triangle. The condition of the roots on the line having 8 parts per million P_2O_5 , and on the line having 8 parts per million K_2O , are about the same as on the 8 parts per million NH_3 line.

Farther within the triangle the general condition of the roots is much better. The solutions in cultures number 25, 32, 33, 34, 42, and 43 show by their roots that the harmful effect of the substance has been somewhat overcome: they are lighter than the others, in fact most of the roots, except the upper and older sections, are white and clear, but the tips are still slightly swollen and somewhat bent.

Another property of the roots which is influenced by harmful bodies is that of root oxidation. It has been shown that plant roots possess a very appreciable power of oxidation, and that this power is stronger in good soils than in poor, or in their extracts, and that harmful bodies retard this oxidation, and beneficial bodies augment it. Fertilizer salts were shown to increase root oxidation, and through this action a reduction in the quantity of the harmful body present was produced.

Some further observations were made in connection with the present investigation in regard to oxidation and the effect of dihydroxystearic acid upon this function. The effect was so marked in the concentrations used, namely 50 parts per million, that the roots at the end of the experiment were found to be almost wholly lacking in ability to oxidize aloin used as an indicator in the manner described in the publication referred to above. Some oxidizing power was still possessed by the cultures in the interior of the triangle, where, as mentioned above, the growth was better than in other regions, although even here the power of oxidation was greatly impaired. Nevertheless, it is interesting to note that the condition which produced the best general development of the plants is closely associated with a power of root oxidation.

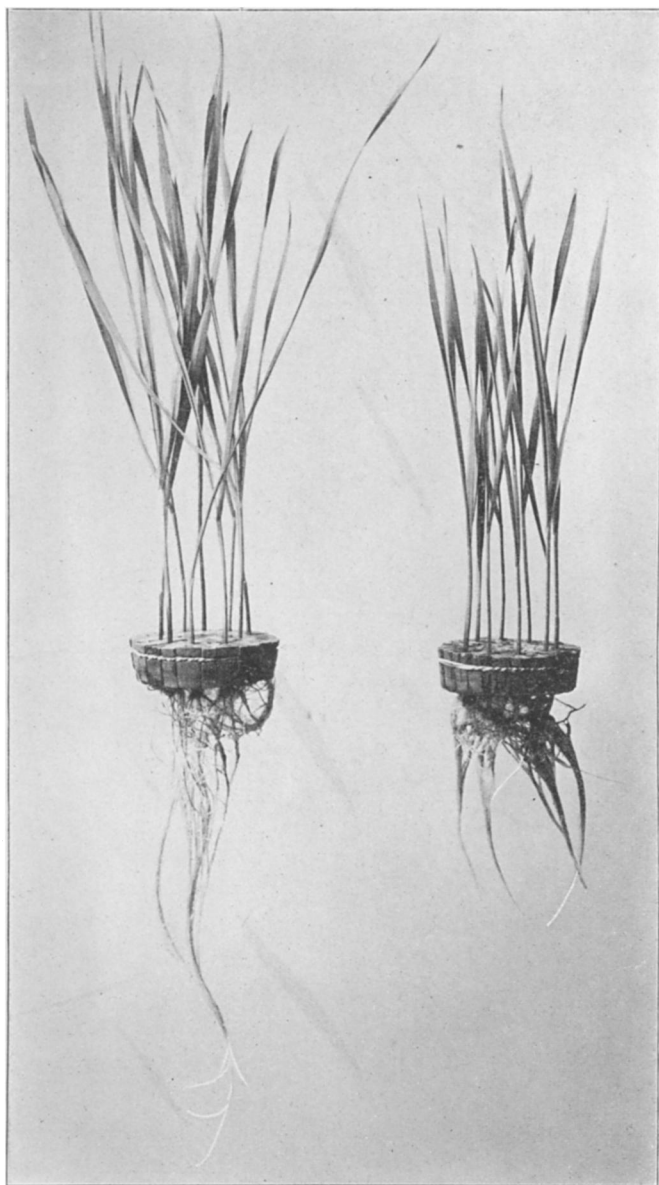


FIG. 3.—Wheat plants growing in a culture solution containing a fertilizer mixture composed of phosphate 60 per cent, nitrogen 20 per cent, potash 20 per cent: 1, without dihydroxystearic acid; 2, with dihydroxystearic acid.

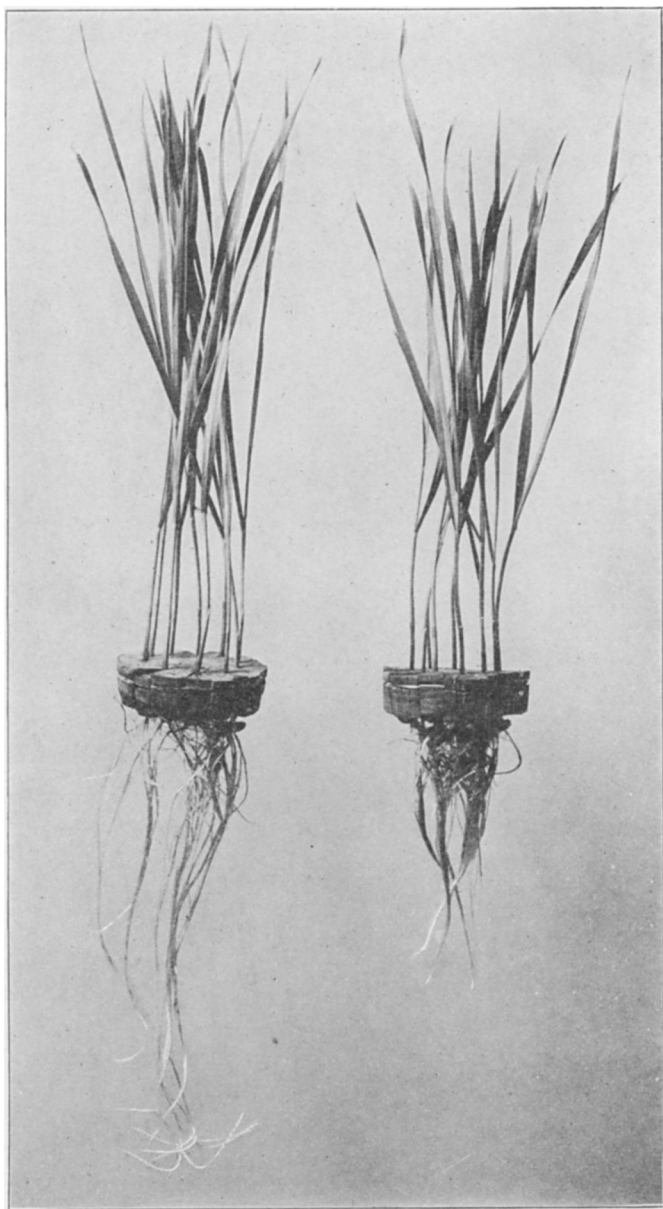


FIG. 4.—Wheat plants growing in a culture solution containing a fertilizer mixture composed of phosphate 20 per cent, nitrogen 60 per cent, potash 20 per cent: 1, without dihydroxystearic acid; 2, with dihydroxystearic acid.

Lower concentrations of dihydroxystearic acid would doubtless be better suited for a more thorough study of this matter.

Some of the plants grown in solutions with and without dihydroxystearic acid are shown in figs. 3, 4, and 5. The cultures taken represent a fertilizer rich in phosphoric acid, one rich in nitrate, and one rich in potash. The results shown in these photographs are representative of the general effect over all the region of the triangle from which they are taken.

GREEN WEIGHT OBTAINED IN THE VARIOUS CULTURES

The green weights obtained in the 66 cultures of the first set are given in the triangular diagram shown in fig. 6. It will be seen that, in harmony with the results given in the previous paper reporting the experiment without the harmful substance, the growth with the single elements or along the lines where mixtures of two occurred was in general less than within the triangle. The region of greatest growth in the solutions without the dihydroxystearic acid was approximately in the middle of the 10 and 20 per cent phosphate lines. In the dihydroxystearic acid cultures it might be said in general that this region of greater growth was displaced along these lines toward the nitrogen side.

The total growth made in the 66 cultures without the dihydroxystearic acid was 207 grams as against 114 grams in the case of the 66 cultures with the 50 parts per million of dihydroxystearic acid, or, putting the first as 100, the latter becomes 55. In other words, the plants with the dihydroxystearic acid made only, as an average of the 66 cultures, a growth of 55 per cent. It will be seen that the harmful substance produced its greatest effect in general along the periphery of the triangle, except, perhaps, along the phosphate-nitrogen line. In the interior of the triangle, where the greater growth occurred under the more normal conditions, the depression caused by the substance was not so marked, and this seems to be especially true in the region nearer to the nitrate end, in which, as already pointed out, the greater growth occurred in the cultures where the dihydroxystearic acid was present.

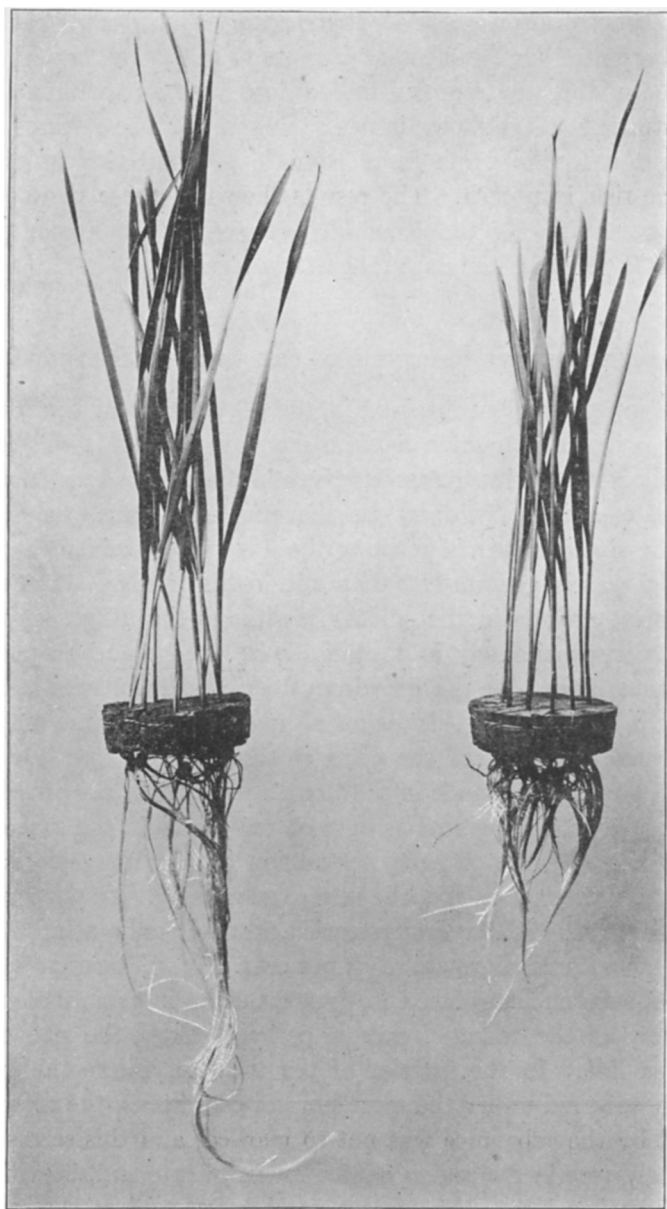


FIG. 5.—Wheat plants growing in a culture solution containing a fertilizer mixture composed of phosphate 20 per cent, nitrogen 20 per cent, potash 60 per cent: 1, without dihydroxystearic acid; 2, with dihydroxystearic acid.

RATIOS OF P_2O_5 , NH_3 , AND K_2O FOUND IN THE VARIOUS CULTURES

In the diagram fig. 8 are given the original ratios of the fertilizer constituents, the ratios left in these solutions as shown by analysis, and the corresponding ratio of the removed constituents, as in the former paper. As before, the large dots in the diagram represent the original ratios according to the scheme previously

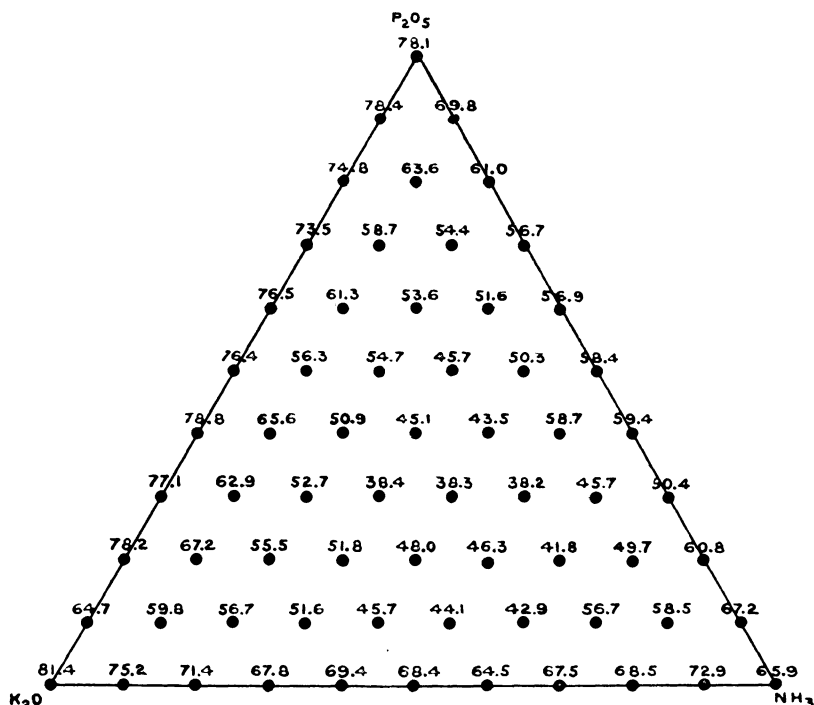


FIG. 7.—Average concentration in parts per million of $P_2O_5 + NH_3 + K_2O$ of the solution after the growth of 10 wheat plants; concentration of original solution was 80 p.p.m.

explained. The circles indicate the ratio left in the solution as shown by analysis, and the other end of the line indicated by an arrow shows the corresponding ratio of the removed materials.

In the former experiment without dihydroxystearic acid there was a decided tendency for these lines to converge toward a region somewhat below the center; that is, the solutions near this central area changed least in their ratio, and the farther the ratios were

removed from this central area originally, the more were they altered in the course of the experiment. This area was between the 10 and 20 per cent phosphate line, and the area of greatest growth occurred in this same region. It is in this region of greatest growth, therefore, that the greater absorption of nutrients took place with the least change in ratio; in other words, the solutions repre-

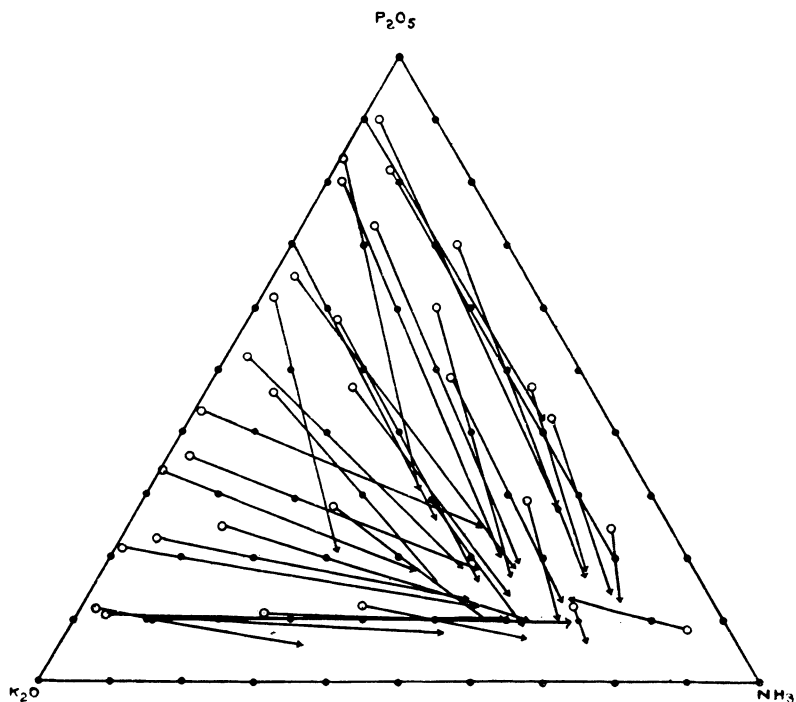


FIG. 8.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5 , NH_3 , and K_2O from the culture solution; the dots indicate the ratio of the constituents in the original solution; the circles show the ratio of the constituents in the solution after growth; and the arrows show the ratio of the decrease.

sented by this region offered the best environment for plant development and the best ratios for the absorption of plant nutrients.

The diagram shown in fig. 8 for the experiment with dihydroxystearic acid shows on the whole a migration of the lines so that they point to an area somewhat nearer to the nitrate end of the triangle. It will be remembered from the data already discussed

that there is a tendency for the area of greatest growth under these conditions to be nearer this end.

In other words, when dihydroxystearic acid is present the results show a tendency for the ratios of the materials removed from the solution to fall nearer to the nitrogen end of the diagram than they do in the case of cultures where this substance is absent. This

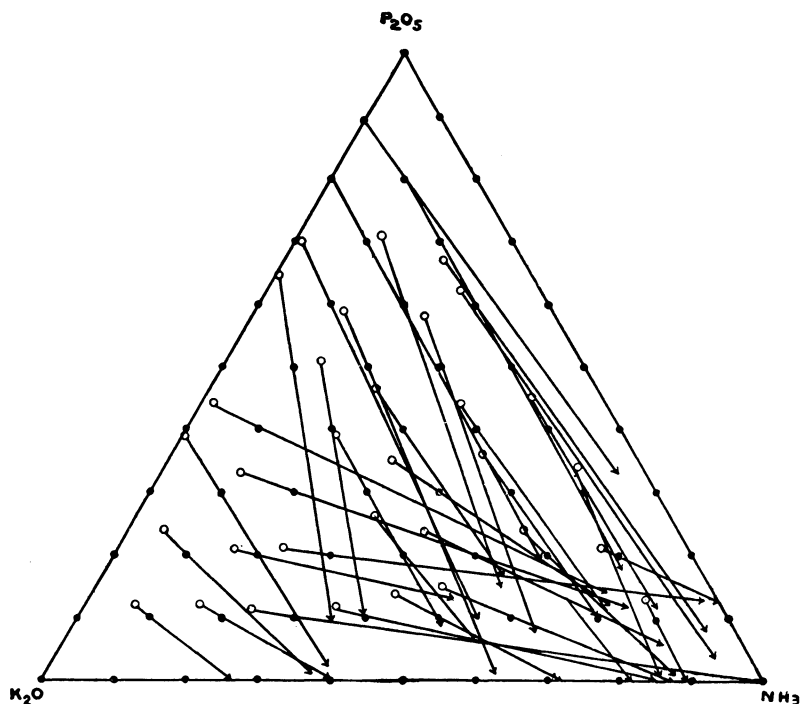


FIG. 9.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5 , NH_3 , and K_2O from the culture solution, in the first period.

tendency is marked from the very first, as is shown by the diagram given in fig. 9 for the first period. Moreover, the points, for instance, of the 10 per cent mixture of P_2O_5 lie very low, and this tendency to lie lower than in the cultures which contained no dihydroxystearic acid is found throughout the experiment, though it is not equally marked in all periods. The average effect has already been given in the diagram fig. 8. The strong tendency

which is shown when this substance is present for a proportionately, and sometimes even absolutely, greater decrease in the nitrogen of the solutions, is strikingly shown in this first period (fig. 9). This varies somewhat from period to period; thus, for instance, in the fourth period, shown in fig. 10, the ratio change is fairly normal, though the usual tendency is seen again in the seventh period, a

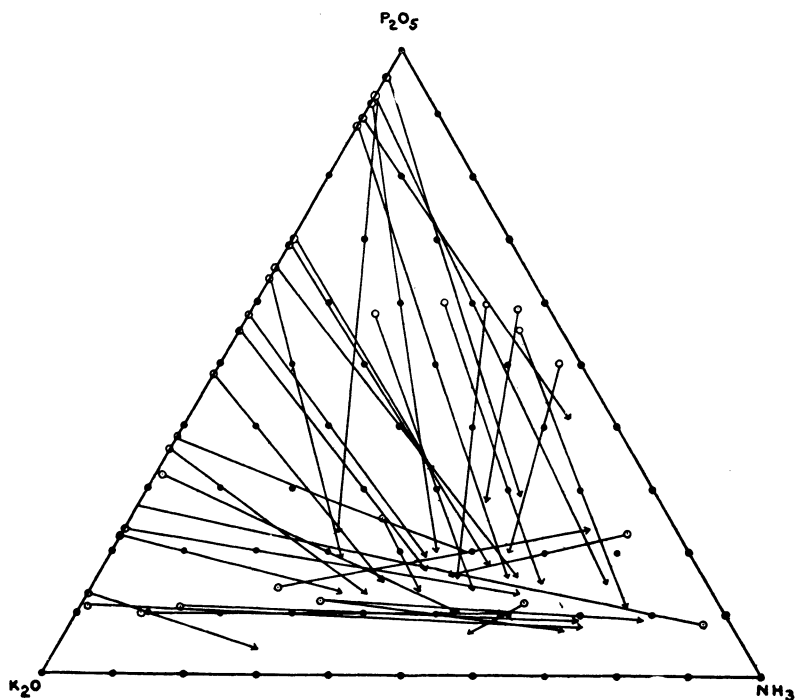


FIG. 10.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5 , NH_3 , and K_2O from the solution, in the fourth period.

diagram of which is presented in fig. 11. These diagrams for the several periods illustrate rather well the general tendencies brought out by an examination of the analytical data. As a rule, beyond the second or perhaps the third period the diagrammatic representation of the result is on the whole uniform, but is influenced undoubtedly by the conditions of growth during any period; in other words, by weather and other conditions, which is shown perhaps quickest

in the nitrate removal from the cultures. The influence of light conditions on different days has already been discussed in the previous paper.

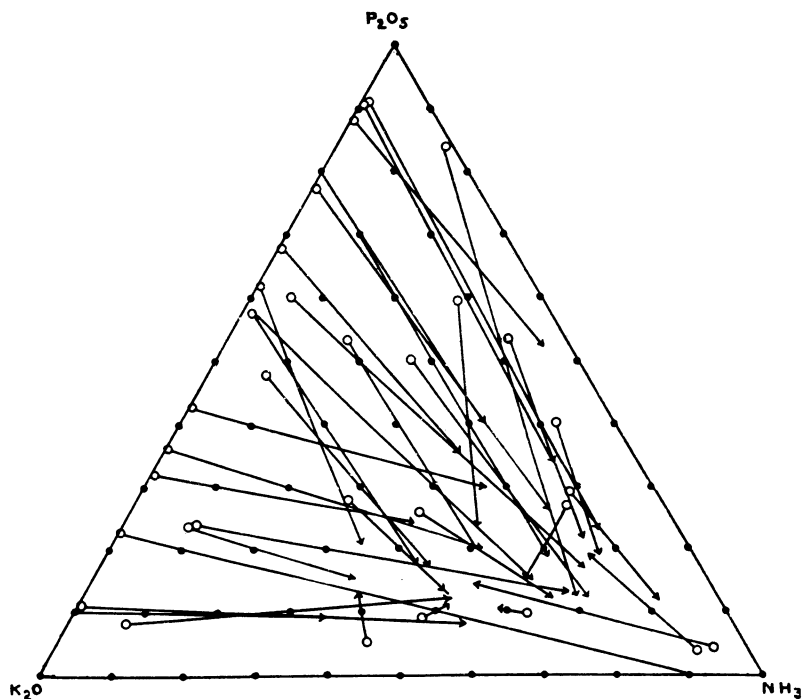


FIG. 11.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5 , NH_3 , and K_2O from the solution, in the seventh period.

THE COMPARATIVE EFFECT PRODUCED ON THE DIHYDROXYSTEARIC
ACID CULTURE SOLUTIONS BY THE FERTILIZER MIXTURES
COMPOSED MAINLY OF P_2O_5 , NH_3 , AND K_2O
RESPECTIVELY

In the experiment presented, as well as in two others in which both sets of cultures were grown simultaneously, there was noticed a more general tendency toward normal development of the plants in the presence of dihydroxystearic acid when the culture solutions contained all three of the fertilizer substances. It was further apparent that there seemed to be a tendency for more normal development in a region nearer to the nitrate end. In order to

trace out this matter, a comparison of the three experiments has been made and is hereby presented. In this way the solutions which contained in all cases 50 per cent or more P_2O_5 are considered in one group; all those which contained 50 per cent or more NH_3 in a second group; and those with 50 per cent or more K_2O in a third group. By grouping the cultures thus, an average result is obtained with the mainly phosphatic, mainly nitrogenous, and mainly potassic fertilizers.

A comparison of the average green weight in each of these groups of cultures was made with those in the corresponding groups in the cultures where dihydroxystearic acid was absent. The relative growths thus obtained, taking the growth without the dihydroxystearic acid as 100, are contained in table I.

TABLE I

SHOWING THE AVERAGE RELATIVE GROWTH MADE IN THE GROUP OF SOLUTIONS WITH DIHYDROXYSTEARIC ACID AND CONTAINING FERTILIZER SALTS HAVING THE COMPOSITION OF 50 TO 100 PER CENT OF ANY ONE OF THE COMPONENTS P_2O_5 , NH_3 , AND K_2O .

EXPERIMENT	RELATIVE GREEN WEIGHT (Green weight without dihydroxystearic acid=100)		
	P_2O_5 50-100 per cent	NH_3 50-100 per cent	K_2O 50-100 per cent
1.....	49	67	43
2.....	60	62	62
3.....	71	68	61

In the first column is given the number of the experiment; in the second column the average relative green weight obtained in the solutions having mainly phosphatic fertilizers; in the third column the same result for the cultures of mainly nitrogenous fertilizers; and in the fourth column the results obtained when mainly potassic fertilizers were present. The results indicate, as was pointed out in discussing the experiment more fully described in this paper, that the mainly nitrogenous fertilizers enabled the plants to make a more normal growth than, on the whole, do the other fertilizers, although with the quantities of salts and harmful substance used in these experiments, the plants were by no means able to overcome the harmful effect entirely, if indeed this is possible.

This general relationship is even more strongly shown when the decreases in the concentrations of the solutions in these various groups are considered. The average decrease, in solutions with and without dihydroxystearic acid, for each group in each experiment, is shown in table II.

TABLE II

SHOWING THE AVERAGE DECREASES IN THE CONCENTRATION OF TOTAL CONSTITUENTS IN THE GROUP OF CULTURE SOLUTIONS CONTAINING FERTILIZER SALTS HAVING THE COMPOSITION OF 50 TO 100 PER CENT OF ANY ONE OF THE COMPONENTS P_2O_5 , NH_3 , AND K_2O , WITHOUT AND WITH DIHYDROXYSTEARIC ACID (ORIGINAL CONCENTRATION = 80 PARTS PER MILLION).

EXPERIMENT	50 100 PER CENT P_2O_5			50-100 PER CENT NH_3			50-100 PER CENT K_2O		
	With-out	With	Decrease in dihydroxy-stearic acid solutions. Normal equals 100	With-out	With	Decrease in dihydroxy-stearic acid solutions. Normal equals 100	With-out	With	Decrease in dihydroxy-stearic acid solutions. Normal equals 100
1.....	27.8	17.6	63	35.4	24.0	68	34.0	13.9	41
2.....	28.9	24.6	55	30.3	34.6	114	38.5	30.8	80
3.....	27.8	23.8	85	35.9	32.0	89	45.0	31.0	69

The third column under each fertilizer group gives the relation between these decreases, the decrease in concentration when dihydroxystearic acid is absent being considered as the normal for comparison and equal to 100. A comparison of these relative effects shows that the mainly nitrogenous fertilizers give the highest results. In other words, the decrease with the nitrogenous fertilizers was more nearly like that observed under the normal conditions where dihydroxystearic acid was absent. This result may be interpreted to mean that the mainly nitrogenous fertilizers decreased the inhibitive effect of the dihydroxystearic acid, although it does not show whether this is a direct or an indirect effect; that is, whether there is an actual decrease of this inhibitive material or whether there is mainly the ability of the plant to withstand the attacks under these conditions. Attention has already been called to the fact that this substance when in soils is most easily destroyed by processes which promote oxidation, and it should be borne in mind that the mainly nitrogenous fertilizers are the ones which promote the most active root oxidation by the plants themselves. A study of the oxidation of roots in these

experiments showed that the dihydroxystearic acid interfered greatly with oxidation, and that this action was overcome to some slight extent in the center of the triangle but nearer to the nitrate end, thus showing perhaps that there is some correlation in these functions.

SUMMARY

The foregoing investigations have given the following results:

1. An organic soil constituent, dihydroxystearic acid, hinders the growth of wheat plants, when this is present in solution in pure distilled water.

2. The compound is also harmful in the presence of nutrient or fertilizer salts in all ratios of the fertilizer elements, P_2O_5 , NH_3 , and K_2O .

3. The compound is more harmful in those ratios of fertilizer elements not well suited for plant growth.

4. The harmful effect of the compound is the least in those ratios of fertilizer elements best suited for plant growth.

5. The compound appears to be relatively much less harmful in the presence of fertilizers mainly nitrogenous than in the presence of fertilizers mainly phosphatic or potassic.

6. The harmful compound modified greatly the removal of fertilizer elements from the solutions. The quantity of phosphate and potash removed was less in the presence of the compound, but the nitrate was not so influenced and on the whole the amount removed was even greater.

7. The compound modified both amount and ratio of the three fertilizer elements removed from solutions, the ratio being higher in nitrogen, which was also the most efficient fertilizer element in decreasing the harmful effect, as above mentioned.

8. The harmful compound has the additional effect of darkening the root tips, stunting root development, causing enlarged root ends, which are often turned upward like fish-hooks, and inhibiting strongly the oxidizing power of the roots.

9. Those fertilizer combinations which tend to increase root oxidation are also the combinations which overcome the harmful effects to the greatest extent.